EXPERIMENTAL STUDIES ON THE DURATION OF LIFE

II. Hereditary Differences in Duration of Life in Line-bred Strains of Drosophila[1]

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Introduction

It was shown in the first paper in this series (27)[2] that there was a marked difference in mean duration of life, and in the form of the l_x curve, between wild-type stocks of *Drosophila* on the one hand and the synthetic quintuple mutation stock on the other hand. It was further made clear that, because of the technique used in the experimental work, there could be no doubt that the basis of this difference must be hereditary and not environmental. Furthermore, Hyde (11) and Pearl (6) have presented evidence for the Mendelian inheritance of this character duration of life.

Given it to be the fact, as the just cited work demonstrates to be the case, that there are hereditary differences within the same species of *Drosophila* in respect of duration of life, the problem which next presents itself is to determine whether *within* a particular strain of *Drosophila* hereditary differences exist, and if so what their magnitude may be, their degree of permanence, etc. In

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[2] A word of explanation is necessary as to the method of handling bibliographic references in this series of papers. In the first paper a list of 26 references numbered consecutively from 1 was appended. It is proposed not to duplicate references in any subsequent paper in the same series. Consequently the first *new* bibliographic citation in the present paper is numbered 27. When any reference is made to titles already cited in the first paper in the series, the numbers which they bear in the list appended to that paper will be used. This practice will be adhered to in all subsequent papers in this series of Studies.

short one wishes immediately to get a kind of knowledge for this organism and character similar to that which Johannsen (28, 29) got for the size character of beans from his pure-line work. The first, and in a sense preliminary, investigations on this problem will be presented in this paper. Later in the series we expect to publish much more extended and penetrating evidence on the same problem. Some, however, must be presented early in the series in order to make the account of subsequent experiments intelligible.

It is obvious that in the case of an organism like *Drosophila* it is impossible to have a pure-line in the strict sense of Johannsen. The most that one can do is to have inbred lines, and the most intense degree of inbreeding possible in the premises is by brother × sister mating. The general plan of the experiments reported in this paper can be outlined as follows:

1. Mate a virgin brother and sister, chosen at random each from the same one of the original 5 foundation stocks (cf. 27).
2. Repeat this for as many pairs as the facilities of the laboratory make possible.
3. Test the progeny of each mated pair separately for duration of life, and form for each group of such progeny a life table.
4. Each such mated pair constitutes the beginning of a line, in which at any time the processes noted under paragraphs 1, 2, and 3 above could be repeated. In this paper will be reported the results of one such repetition.

The general technique of the experimental work has been fully described in the first paper of this series and need not be repeated. It should merely be emphasized again that the environmental conditions in respect of food, housing, temperature (25° C.) and atmospheric conditions were identical for all the flies in the experiments here reported.

Duration of Life in Different Progeny Groups out of Brother × Sister Matings

The survivorship data (l_x frequencies) for 7 progeny groups each out of a mating of brother × sister are exhibited in Table II. All distributions are put on the same basis of 1,000 flies at emergence from the pupal stage. The absolute numbers of flies involved in each experiment are given at the foot of each column. These numbers are

TABLE I

Brother × Sister Matings. First Test

Lines	Original Stock (Described in (27))	Date of Mating	Date of Emergence
100.....	Old Falmouth	April 8, 1920	April 19–May 3
101.....	" "	April 7, 1920	April 17–May 2
201.....	New Falmouth	April 7, 1920	April 18–May 2
202.....	" "	April 10, 1920	April 20–April 29
300.....	Sepia	April 7, 1920	April 17–May 3
301.....	"	April 6, 1920	April 17–May 3
303.....	"	April 8, 1920	April 18–May 2

TABLE II

Survivorship Distributions of Progeny of Brother × Sister Matings. Both Sexes Together

Age in Days	Numbers of Survivors up to Indicated Age in Lines No.						
	100	101	201	202	300	301	303
1	1,000	1,000	1,000	1,000	1,000	1,000	1,000
6	983	993	1,000	689	926	870	882
12	937	987	1,000	607	858	727	764
18	891	934	952	492	809	602	702
24	811	901	943	426	623	441	621
30	743	875	857	344	549	342	522
36	589	855	790	197	383	255	429
42	514	770	600	148	272	205	311
48	406	599	505	148	148	130	261
54	240	493	381	49	105	75	168
60	91	296	219	33	12	31	112
66	29	99	133	16	6	12	56
72	6	20	10	0	0	2	0
78	0	7	10	—	—	0	—
84	—	7	0	—	—	—	—
90	—	0	—	—	—	—	—
Abs. No. of flies	175	152	105	61	162	161	161

smaller than is desirable, but these experiments represent a relatively early stage of the work before the technique of getting maximum progenies for life table work had been perfected. Further it must be remembered that the individuals in any column are the progeny of only one single pair of parents. The source of the lines together with other pertinent data are shown in Table I.

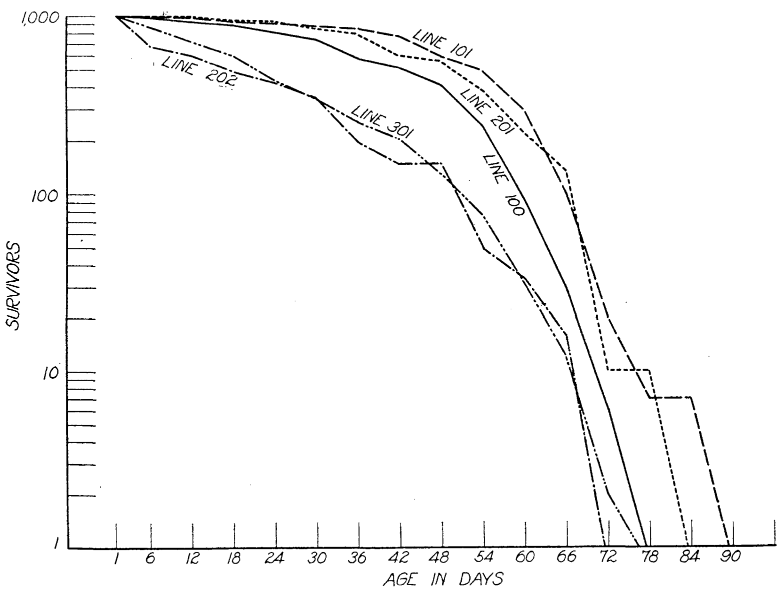

FIG. 1. Survivorship (l_x) graphs for lines 100, 101, 201, 202 and 301.

Five of these distributions are shown graphically in Fig. 1, and their biometric constants are given in Table

TABLE III

FREQUENCY CONSTANTS FOR d_x DISTRIBUTIONS. FIRST TEST

Line No.	Mean Duration of Life (Days)	Standard Deviation (Days)	Coefficient of Variation
100.....	40.45 ± .84	16.38 ± .59	40.49 ± 1.68
101.....	50.02 ± .85	15.51 ± .60	31.01 ± 1.31
201.....	47.40 ± .99	15.03 ± .70	31.71 ± 1.51
202.....	22.04 ± 1.57	18.18 ± 1.11	82.49 ± 7.74
300.....	31.19 ± .83	15.76 ± .59	50.53 ± 2.33
301.....	25.28 ± .92	17.25 ± .65	68.24 ± 3.56
303.....	32.02 ± 1.07	20.04 ± .75	62.59 ± 3.14

III. In calculating these constants, the absolute d_x frequencies, and not the per mille frequencies, were of course used.

From these data it is at once apparent that these progeny groups show distinct, and in some cases decidedly large, differences both in mean duration of life and in the form of the mortality distributions. Lines 101 (Old Falmouth stock) and 201 (New Falmouth stock) show the longest mean duration of life, and they are sensibly identical in the form of the life curve, having regard to the errors of random sampling. The difference in the means for these two lines is 2.62 ± 1.31 days, an obviously insignificant difference, only 2 times its probable error. Similarly these two lines do not significantly differ in absolute or relative variability, the difference between the standard deviation being $.48 \pm .92$.

Line 100 (Old Falmouth stock) has a distinctly and significantly lower mean duration of life than 101 or 201. Comparing it with line 101 the difference in the means is 9.57 ± 1.20 days or approximately 8 times its probable error. The l_x curve lies throughout its course below the lines for 101 and 201. Line 100 is also relatively more variable in duration of life than 101 and 201, but largely because of the difference in the means.

The individuals in line 202 (New Falmouth stock) are the shortest lived of any here dealt with, and the shortest-lived wild-type strain we have as yet isolated. Its mean duration of life is less than half that shown by lines 101 and 201 and only a little more than half that of line 100. Line 202 shows the highest relative variability in duration of life of any of the lines here discussed. It also has the highest absolute variability with one exception (line 303).

Lines 300, 301 and 303 (Sepia stock) are all relatively short-lived lines. 300 and 303 are substantially identical, while 301 has a lower mean approaching that of line 202. These sepia lines are also characterized by high relative variability.

Results of Inbred Re-tests for Constancy

During the progress of the experiments described in the preceding section the offspring flies (from original brother × sister matings) in each of the lines, whose duration of life was being tested, were allowed to mate at random in their bottles, and their progeny removed to form stocks of the several lines. These stocks were allowed to reproduce in stock bottles, all matings being therefore random *within the line,* for a period of about 7 months (cf. Table IV). At the end of that time it was decided to make a re-test of each line to see how it was then behaving relative to duration of life. There was then made, at dates indicated in Table IV, a random selection from each line stock bottle from which a brother and sister pair was bred, and these two individuals were mated to get a set of progeny on which to carry out a second set of life duration experiments. The necessary facts as to line numbers and dates on this re-test are given in Table IV.

TABLE IV

Brother × Sister Matings. Second Test

Line from which Second Selection of Brother and Sister Was Made	Number of Line of Progeny of Second Brother × Sister Mating	Date of Original Brother × Sister Mating	Date of Second Brother × Sister Mating
100	104	April 8, 1920	November 6, 1920
101	107	April 7, 1920	October 14, 1920
201	207	April 7, 1920	October 18, 1920
202	208	April 10, 1920	October 14, 1920
300	304	April 7, 1920	November 6, 1920
301	307	April 6, 1920	October 14, 1920
303	309	April 6, 1920	October 14, 1920

The survivorship distributions of the progeny groups of this second brother × sister mating are given in Table V, and the biometric constants calculated from the observed d_x distributions in Table VI. These tables are for comparison with Tables II and III above.

TABLE V

SURVIVORSHIP DISTRIBUTIONS OF PROGENY OF SECOND BROTHER × SISTER MATINGS. BOTH SEXES TOGETHER

Age in Days	Numbers of Survivors up to Indicated Age in Lines No.						
	104	107	207	208	304	307	309
1	1,000	1,000	1,000	1,000	1,000	1,000	1,000
6	997	1,000	973	833	1,000	862	1,000
12	923	950	926	738	870	700	978
18	871	926	819	643	870	623	911
24	713	917	792	595	674	469	700
30	629	901	785	500	478	392	489
36	552	860	711	286	435	285	456
42	469	777	644	167	261	177	267
48	395	686	530	0	152	92	89
54	304	595	430	—	109	46	67
60	178	488	255	—	0	23	44
66	66	264	141	—	—	8	0
72	0	83	20	—	—	8	—
78	—	8	20	—	—	8	—
84	—	0	7	—	—	0	—
90	—	—	0	—	—	—	—
Abs. No. of flies	286	121	149	42	46	130	90

TABLE VI

FREQUENCY CONSTANTS FOR d_x DISTRIBUTIONS. SECOND INBRED TEST

Line No.	Mean Duration of Life (Days)	Standard Deviation (Days)	Coefficient of Variation
104	39.59 ± .74	18.63 ± .53	47.06 ± 1.62
107	53.74 ± 1.07	17.40 ± .75	32.38 ± 1.54
207	45.34 ± 1.10	19.97 ± .78	44.04 ± 2.03
208	25.65 ± 1.53	14.68 ± 1.08	57.23 ± 5.42
304	32.09 ± 1.43	14.43 ± 1.01	44.97 ± 3.75
307	25.22 ± .99	16.70 ± .70	66.22 ± 3.79
309	33.00 ± .91	12.84 ± .65	38.91 ± 2.23

The purpose of this second test was, of course, to see to what extent duration of life was holding constant in the line. During the period between the first and second test the stocks of the several lines had been subjected to varying environmental influences, in particular in relation to temperature, the stock bottles having been kept at room temperature, which varied rather extensively. Did the lines after 7 months have the same characteristic life curves that they exhibited on the first test? Allowing 12

days from generation to generation in the case of flies reproducing freely at random in stock bottles, the interval elapsing between the first and second tests would cover roughly almost 18 generations. This is a long period and affords abundant opportunity for change in the average genetic constitution of the population.

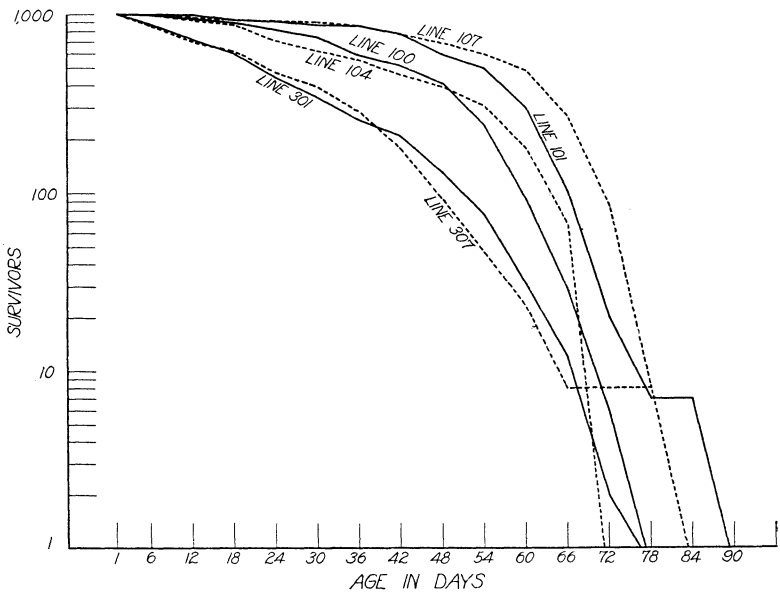

FIG. 2. Comparing the l_x lines of the first and second inbred tests. of lines 101, 100 and 301.

An examination of Tables V and VI and Fig. 2 shows at once, in a general way, that the characteristic features of the several lines in respect of duration of life did in fact hold remarkably constant during this period. A more precise comparison of the means is made in Table VII.

There can be no question of the substantial constancy of these lines, over the period covered in the tests in respect of duration of life. The l_x curves run well together till the upper end of life is reached, where, because of the small numbers involved, there is some irregularity. In no case is the difference between two comparable means, as shown in Table VII, as much even as three times its prob-

able error, nor is there any certainly significant change in variability having regard to the probable errors of the differences involved.

TABLE VII

DIFFERENCES IN MEAN DURATION OF LIFE BETWEEN THE FIRST AND SECOND INBRED TESTS OF THE SEVERAL LINES

Corresponding Lines (Mean of Second Test Minus Mean of First)	Difference of Means (Days)	$\frac{\text{Diff.}}{\text{P. E. Diff.}}$
104–100	$-\ .86 \pm 1.12$	.77
107–101	$+3.72 \pm 1.37$	2.72
207–201	-2.06 ± 1.48	1.39
208–202	$+3.61 \pm 2.19$	1.65
304–300	$+\ .90 \pm 1.65$	.54
307–301	$+\ .06 \pm 1.35$	.04
309–303	$+\ .98 \pm 1.40$	.70

RESULTS OF MASS CULTURE RE-TESTS FOR CONSTANCY

The point may well be made that in the re-tests of the lines described in the preceding section an additional element is introduced in the fact that the flies for the re-test were the progeny of a second brother $\times$ sister mating. What one wishes to know is: what degree of constancy in duration of life is exhibited by the general stocks in each line, mating purely at random, after the initial selection and inbreeding? We wish now to present some data on this point. Table VIII gives the biometric constants for this material. Mass culture re-tests have been made on two of the original lines, 100 and 101. These mass culture re-tests were made in two ways as follows:

(*a*) From the stock bottles of the line to be tested a large sample of progeny was taken at random each day as the flies emerged from the pupal stage, and these progeny flies were put in small bottles for a duration of life experiment in the usual way described in (27).

(*b*) From the stock bottles of a particular line to be tested a number of virgin flies (usually 8 to 10 of each sex) were taken at random immediately upon emergence, and mated as a group in a mating bottle. The progeny from this sample was then removed, upon emergence, to small bottles and a regular duration of life test carried as described in (27).

TABLE VIII

FREQUENCY CONSTANTS FOR MASS CULTURE RE-TESTS. ORIGINAL LINES

Line No.	Test	Dates of Emergence	Numbers of Flies	Mean (Days)	Standard Deviation (Days)	Coefficient of Variation
100	Mass Culture	1920 Sept. 25–Oct. 21	433	33.05 ± .76	23.48 ± .54	71.05 ± 2.31
	Original brother × sister	1920 Apr. 19–May 3	175	40.45 ± .84	16.38 ± .59	40.49 ± 1.68
Difference		5 mo. 12 days	—	− 7.40 ± 1.13	+ 7.10 ± .80	+30.56 ± 2.68
101	Mass Culture *A*	1920 Sept. 25–Oct. 21	473	53.09 ± .70	22.53 ± .49	42.44 ± 1.09
	Mass Culture *B*	1921 Mar. 18–Apr. 4	124	48.53 ± 1.02	16.76 ± .72	34.54 ± 1.65
	Original brother × sister	1920 Apr. 17–May 2	152	50.02 ± .85	15.51 ± .60	31.01 ± 1.31
Difference *OA*		5 mo. 13 days	—	+ 3.07 ± 1.10	+ 7.02 ± .77	+11.43 ± 1.70
Difference *OB*		10 mo. 29 days	—	− 1.49 ± 1.33	+ 1.25 ± .94	+ 3.53 ± 2.11

It is at once apparent that the mass re-tests on line 101 gave extremely satisfactory results as to constancy of duration of life in the line, after intervals of approximately 5 and 11 months. The mean value for either the *A* or the *B* test does not significantly differ, having regard to its probable error, from the mean shown on the original test at the start of the line. The mean of the *A* mass re-test almost exactly agrees with that of the second inbred test of the same line, as given in Table VI.

In the case of line 100, the mass re-test after $5\frac{1}{2}$ months approximately does not give such close agreement. The mean is significantly lower, the difference being 6.6 times its probable error. No explanation of this result is, as yet, forthcoming, but it probably means no more than lack of genetic purity in the line. It is, however, interesting to note that the sense of the change is in the same direction as that in which line 100 in general differs from line 101, which we regard as our most typical wild-type line in respect of duration of life. That is, line 100 is, as compared with 101, a shorter-lived line. Its mass culture re-test is still shorter lived.

The variability in respect of duration of life, whether measured in absolute or relative terms, is uniformly higher and in two cases out of the three by a significant amount in the mass culture than in the original inbred tests. This is, of course, exactly what would be expected on general genetic grounds. One brother $\times$ sister mating, as has been shown by Pearl (30), Jennings (31) and others, reduces the heterozygosis in the strain by only 50 per cent. It is interesting to note, in connection with the explanation suggested above for the difference in the means in the case of line 100, that the variability in the mass re-test on that line is very much higher than in the original inbred test.

A mass re-test was carried out on two of the lines from the second brother $\times$ sister matings. The results from these experiments are presented in Table IX.

TABLE IX

Frequency Constants for Mass Culture Re-tests. Twice Inbred Line

Line No.	Test	Date of Emergence	Numbers of Flies	Mean (Days)	Standard Deviation (Days)	Coefficient of Variation
107	Mass Culture *A*	1921 Apr. 19–Apr. 22	1,338	49.74 ± .25	13.69 ± .18	27.52 ± .54
107	Second brother × sister *S*	1920 Oct. 25–Nov. 4	121	53.74 ± 1.07	17.40 ± .75	32.38 ± 1.54
101	Original brother × sister *O*	1920 Apr. 17–May 2	152	50.02 ± .85	15.51 ± .60	31.01 ± 1.31
Difference *SA*		6 mos. 9 days	—	− 4.00 ± 1.10	− 3.71 ± .77	− 4.86 ± 1.63
Difference *OA*		11 mos. 27 days	—	− .28 ± .88	− 1.82 ± .96	− 3.49 ± 1.42
309	Mass Culture *A*	1921 May 18–25	468	34.04 ± .34	11.02 ± .24	32.38 ± .79
309	Second brother × sister *S*	1920 Oct. 25–Nov. 1	90	33.00 ± .91	12.84 ± .65	38.91 ± 2.23
303	Original brother × sister	1920 Apr. 18–May 2	161	32.02 ± 1.07	20.04 ± .75	62.59 ± 3.14
Difference *SA*		6 mos. 28 days	—	+ 1.04 ± .97	− 1.82 ± .69	− 6.53 ± 2.37
Difference *OA*		13 mos. 1 day	—	+ 2.02 ± 1.12	− 9.02 ± .79	−30.21 ± 3.24

The substantial constancy of line 101, in both mass and inbred tests, is evident. In respect of variability the line behaved somewhat like 303 discussed below.

In line 303 again the constancy of the line in respect of mean duration of life is as definite as could be expected. Over periods of approximately 7 and 13 months, the mean duration of life has not sensibly changed, having regard to the probable error involved. The results respecting variability are somewhat anomalous. Both the second inbred and the mass re-test show variability of a distinctly lower order than was exhibited by the progeny of the original brother × sister mating. It seems probable that the original test by accident gave a variability result higher than was really characteristic of the line. But the mass culture re-test exhibits a lower variability, not certainly significant, to be sure, than the first test on line 309. Of course it is to be expected that with continued brother × sister mating the variability of mass cultures from the line would come nearer and nearer to that of a further inbred lot of progeny from the same line. Probably the results of Table IX are an expression of the realization of such expectation, obscured by the fact that the numbers are small and the errors of sampling consequently relatively large.

Discussion and Summary

The data presented in this paper appear to demonstrate, with comprehensiveness and accuracy, three broad facts.

A. That there exist in a general population of *Drosophila melanogaster* (or its mutants) genetic differences in respect of duration of life.

B. That these genetic differences are capable of isolation, by appropriate selection and inbreeding.

C. That within an even moderately inbred line, the genetic differences in duration of life remain constant over periods of at least 10 to 25 or more generations.

These facts, based upon the determination experimentally of the duration of life of 3,039 individual flies in 18 experiments, under constant environmental conditions, place this character "duration of life" in the category of genetically definite and workable characters, and indicate that it will just as well repay careful analytical study as the characters more usually dealt with. Furthermore, duration of life is a character of great general biological significance.

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